

# Department of Defense Legacy Resource Management Program

PROJECT NUMBER 08-401

Establishing American chestnut test orchards on two TNARNG installations: contributing to the efforts to restore an ecological and cultural giant to the forest ecosystems of the eastern United States

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October 23, 2009

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1. REPORT DATE 23 OCT 2009	2. REPORT TYPE		3. DATES COVE	red to 00-00-2009	
4. TITLE AND SUBTITLE			5a. CONTRACT NUMBER		
Establishing American Chestnut Test Orchards on Two TN Army National Guard Installations: Contributing to the Efforts to Restore an Ecological and Cultural Giant to the Forest Ecosystems of the Eastern United States			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND AI Ofc Deputy UnderSecretary of Defense Environment,Department of Defense I Program,3400 Defense Pentagon,Wash	se for Installations & Legacy Resource Ma	U	8. PERFORMING REPORT NUMB	G ORGANIZATION ER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT <b>Approved for public release; distribut</b>	tion unlimited				
13. SUPPLEMENTARY NOTES					
14. ABSTRACT					
15. SUBJECT TERMS					
16 SECURITY CLASSIFICATION OF		17 LIMITATION OF	18 NUMBER	19a NAME OF	

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unclassified

a. REPORT

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b. ABSTRACT

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**Report Documentation Page** 

RESPONSIBLE PERSON

Form Approved OMB No. 0704-0188

### Final report on Legacy Project #08-401:

Establishing American Chestnut Test Orchards on Two TN Army National Guard Installations:

Contributing to the Efforts to Restore an Ecological and Cultural Giant
to the Forest Ecosystems of the Eastern United States

#### **Introduction**

After attending a presentation in November 2006 on the American chestnut breeding program, led by the president of the Tennessee chapter of the American Chestnut Foundation (TACF), personnel from the Environmental Branch (ENV) of the Tennessee Army National Guard (TNARNG) began looking for ways that the TNARNG could contribute to the restoration effort. In the following months, Janie Becker, a biologist for the TNARNG, attended local TACF meetings, made contacts with area chestnut growers, and began to work out the logistics of establishing American chestnut backcross orchards on TNARNG properties.

One of the most essential resources needed for the backcross program is land for the seed orchards where the hybrid chestnuts can be grown. The Tennessee Army National Guard (TNARNG) has small areas on its training sites that are not actively used for military training, and so a cooperative agreement was developed with the American Chestnut Foundation (TACF) to establish American chestnut seed orchards at two of its facilities.

TNARNG provided the land and oversight of the project. TACF provided expertise and plant material (seeds and seedlings). As it was known that the cost of orchard set-up materials, maintenance supplies and fence construction would be significant, TNARNG applied for FY 2008 funding through the DoD Legacy Resource Management Program and was awarded funds in January 2008. The Legacy Program is dedicated to supporting efforts to preserve and enhance the natural and cultural heritage of DoD facilities, while maintaining military readiness.

Because the orchards were to be direct seeded and chestnut seeds germinate and survive best when planted by early March, there was not enough time to prepare the sites for planting in the first year of Legacy funding. It was determined that orchard preparation would be conducted during the winter of 2008/2009 and that the orchards would be planted in the early spring of 2009. A request for a No-Cost Extension was submitted in February 2008 and granted shortly thereafter.

#### The Backcross Method

Several different approaches are being taken in the effort to restore American chestnut (*Castanea dentata*). One of these, the backcross method, seeks to instill resistance to the chestnut blight (*Cryphonectria parisitica*) into American chestnuts by initially hybridizing 100% American chestnuts with blight resistant Chinese chestnut (*Castanea mollissima*) and then conducting a series of backcrosses with 100% American chestnut trees (See Figure 1). Within each generation, only trees exhibiting both blight resistance and phenotypically American chestnut traits are used to produce seed for the following generation. Most of the backcross individuals planted on TNARNG orchards are of the 3<sup>rd</sup> backcross generation (BC3). Once BC3 trees reach reproductive age (usually at 5-7 years), blight-resistant individuals are intercrossed with other BC3 trees, producing the BC3F2 generation. Another intercross between two BC3F2 individuals produces the BC3F3 generation; this is the final cross in this breeding design. It is expected to yield highly blight-resistant trees that will be used in large-scale forest test plantings.

## THE AMERICAN CHESTNUT FOUNDATION **BACKCROSS BREEDING PROGRAM** ADDITIONAL AMERICAN CHESTNUT CHARACTERISTICS ARE REGAINED WITH EACH BACKCROSS TACF expects a high level of blight resistance and American characteristics to be present in selected $BC_3F_2$ seed orchard parents. Their $BC_3F_3$ progeny will be extensively tested by TACF for blight resistance and ability to compete in the forest. Chinese American - This cross produces on F1 f American — This is the first backcross to the American and produces an $BC_1$ American - This is the second backcross to the American and produces a BC<sub>2</sub> American - This is the third backcross to the American and produces a BC<sub>3</sub> This is the first intercross and and produces a BC<sub>3</sub>F<sub>2</sub> BC, BC3F2 - This is the second intercross and produces a BC<sub>3</sub>F<sub>3</sub> This is the final intercross. and is expected to show a high level of blight resistance in initial forest test-plantings. Note: In each step, the backcross is selected for resistance. Trees indicate average fraction of American genes with no selection.

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Figure 1 The backcross breeding process (chart courtesy of The American Chestnut Foundation)

The American Chestnut Foundation provided all seeds used in TNARNG orchards. American chestnut Backcross 2 (approximately 7/8 American chestnut) and Backcross 3 (approximately 15/16 American chestnut) comprise over 90% of the trees in the orchards. The remainder of trees planted are controls including: F1 generation chestnuts (50% American, 50% Chinese), 100% American chestnuts, 100% Chinese chestnuts, and, at VTS-Catoosa, 100% Allegheny chinquapins (*Castanea pumila*), a close relative of American chestnut that exhibits some susceptibility to the blight, but has not been affected to the same degree as its cousin.

#### **Site Selection**

The TNARNG Volunteer Training Site-Catoosa, in Tunnel Hill, Georgia (Figure 2), was an obvious selection for an American chestnut orchard as it is located within the native range of the American chestnut. This 1,628 acre facility lies in the Ridge and Valley province in the foothills of the Appalachian Mountains and was likely an excellent habitat for American chestnut prior to the arrival of the blight. Its slopes are well drained and have sandy, slightly acidic soils.

A second orchard location was chosen at VTS-Milan, in Lavinia, TN (Figure 3). VTS-Milan is at the extreme western edge of American chestnut's native range in Tennessee, but as the TNARNG's second largest installation at 2,470 acres, it offered more flexibility in size and location options for an orchard site than other TNARNG facilities to the east, such as Readiness Centers. In addition, the American chestnut orchard project has received an enthusiastic response at VTS-Milan since it was first proposed, with installation managers and staff expressing a keen interest in the restoration effort and its implications for military installations in the region. With the exception of the bottomlands adjacent to Johns Creek and Halls Branch, the facility consists mostly of broad uplands on gently sloping topography. These upland soils are generally well-drained to moderately well-drained.

Other sites, including a number of TNARNG Readiness Centers, were considered for American chestnut orchard sites because of their soil types, habitat, and geographical setting. However, it was determined that the limited onsite personnel could be a detriment to the success of an orchard if located at a Readiness Center.

#### Site preparations at VTS-Catoosa

The orchard site selected at VTS-Catoosa is approximately one acre in size and is situated on a south-facing slope within a heavily wooded, 30-acre area at the north end of the training site. This portion of the facility is separated from the main training site by a public road and is seldom used for training operations.

Soil samples were collected from the proposed orchard site and sent to the University of Tennessee Soil, Plant, and Pest Center for routine analysis of pH as well as recommended rates of application of fertilizer and other amendments.

In preparation for the orchard, over 80 trees of 6 inches DBH or greater were cleared, cut flush to the ground, and treated with herbicide to minimize resprouting. Clearing was conducted in January and February of 2009 using a combination of TNARNG soldiers and ENV personnel. Most trees greater than 3 inches DBH were cut using chainsaws, delimbed, and either cut into firewood for troop use, or left in 8-10 foot sections and set aside for future ENV projects. Smaller trees and shrubs were cut using a forestry mower. All slash was either burned onsite or mulched using the forestry mower. The entire clearing

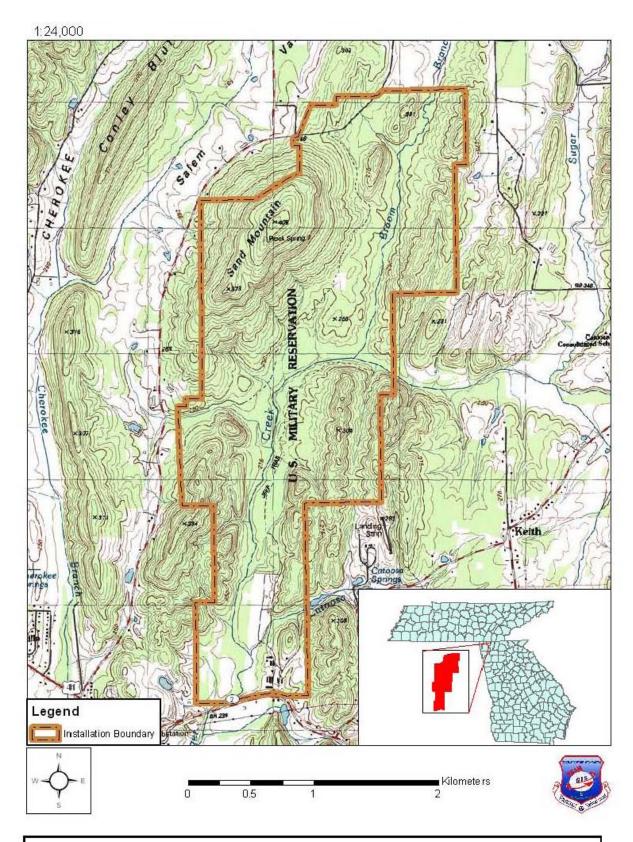


Figure 2 Regional setting and local topography of VTS-Catoosa

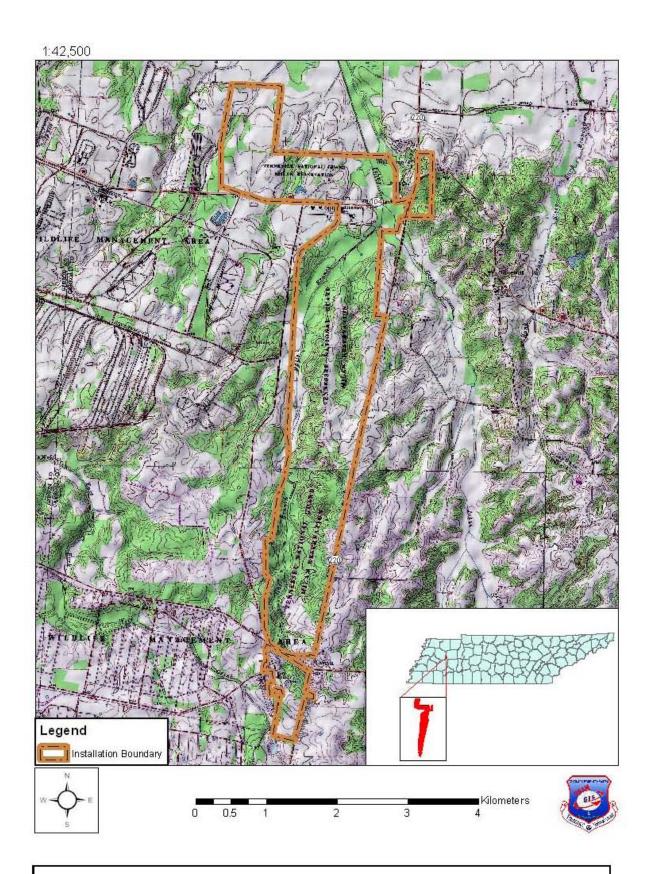


Figure 3 Regional setting and local topography of VTS-Milan

process took nearly three weeks, using an average of 5 persons on site per day. This period did include travel time to and from Nashville, poor weather conditions, equipment malfunctions, and the use of workers who were (generally) unaccustomed to this type of labor. Using a professional tree clearing service, and barring inclement weather, this operation would probably take approximately one week.

Once the orchard site was cleared of trees, construction of the 8-foot tall perimeter fence began. Wooden corner and line posts were installed in mid-March, 2009. Treated pine 4x4 inch posts were used for line posts and corner posts, while 6x6 inch posts were employed as hinge posts for the orchard's two gates. Postholes were dug using a 12-inch hydraulic auger. All wooden posts were set with concrete. After attaching tensioning wire to brace posts and corner posts, it was determined that it would have been advisable to use larger posts in the corners, as the stress of the wire (and later, the woven wire fence) was enough to bow the posts, and in some cases, risk breakage. This was especially true in the southwest corner of the orchard that is near a natural drainage and often has saturated soils which allowed the corner post to shift and lean. To alleviate this problem, poles from the previously harvested timber were used to prop up corner posts from the inside of corners, or, in the case of the wet, southwest corner, a much larger post (~10 inches DBH) was installed directly behind the corner post and used to anchor the unstable corner post using bracing wire.

An eight-foot tall fence was achieved by using two rows of 47-inch tall woven wire. Ten-foot galvanized steel t-posts were placed every 10-12 feet between brace posts and driven 2 feet into the ground. The fence was stretched using a combination of ratchet straps anchored to large, nearby trees and/or to the bucket of a front-end loader. Fence construction took approximately three and a half days using three TNARNG soldiers and two ENV personnel.

The orchard was laid out so that rows would run roughly west to east, perpendicular to the main slope. A 30-foot buffer around the orchard's inner perimeter was created to allow for maneuvering of equipment without the risk of running over chestnut trees located at the very ends of the rows. The rows were spaced 15 feet apart and trees within rows 7 feet apart from each other. This close spacing is standard of backcross orchards in which many trees are culled out over the years as they prove susceptible to the chestnut blight.

Soils in any orchard setting must be uncompacted so that seedlings and trees will be able to extend their roots easily and so that water can penetrate deeply into the soil. The ground at the site was determined to be moderately compacted by the equipment used in the tree clearing and was in need of some form of loosening in the planting areas. A ditch digger was available from the training site and the original idea was to lay out the rows with survey flags, and use the ditch digger to make a trench down each row approximately 6-8 inches wide by 3-4 feet deep. After trying this on Row 1 of the orchard, it became apparent that this was not a feasible method due to the amount of time it took (~2 hours for a 115 foot row). The training site offered to instead drill each tree hole with their 18-inch auger. This took considerably less time (~45 minutes per 115 feet) and provided a larger and better prepared seedbed for each tree. The auger penetrated the soil 3-4 feet deep and left most of the tilled soil inside the hole. Any soil that was pulled out by the auger was generally deposited in a ring immediately surrounding the freshly dug hole and was simply raked and/or shoveled back to the hole. Because the soil was loosened by the auger, the dirt overfilled each hole and was mounded at each tree position where it then settled over time. Using the auger method to drill tree holes, soil preparation for the whole orchard was completed in less than a day and a half, using one auger operator and one spotter on the ground to line up the auger to the hole.

#### Site preparations at VTS-Milan

The backcross orchard at VTS-Milan is located on the northern end of the installation and is bordered to the south by State Highway 104. The site is a triangularly-shaped field that is nearly 3 acres in size. Once this location was selected, it was not expected to be planted with chestnuts to its full capacity because of poor drainage in the southeastern corner of the plot. For many years, training site personnel have maintained this field, dominated by tall fescue (*Schedonorus phoenix*) and broomsedge (*Andropogon virginicus*), with seasonal bushhogging. There has been no historical use of the field for military training.

Two 100% American chestnut seedlings were set out at the Milan field in early 2008 in order to test the site's soils for the fungal pathogen *Phythopthora cinnamomi*. *P. cinnamomi* is a soil-borne water mold that invades root tissues of many plants, absorbing the root's nutrients and carbohydrates, causing the root to rot and preventing water and nutrient uptake by the rest of the plant. It is a serious problem for chestnut growers, especially in the southeast. American chestnut breeders are starting to select for resistance to *P. cinnamomi* in addition to resistance to the chestnut blight. Planting these two seedlings was a good test to determine if soils in the plot contained the pathogen because, if present, the trees would likely die or show considerable signs of deterioration within the first growing season. As the seedlings appeared to remain healthy throughout their first year in the proposed orchard site, it was determined that the site was most likely free of *P. cinnamomi*. As a secondary precaution, however, soil samples were collected from locations throughout the field and sent to the Soils and Plant Sciences Laboratory at Clemson University for culture; *Phythopthora* was absent from all cultures.

Soil samples were also collected and sent to the University of Tennessee Soil, Plant, and Pest Center for routine analysis of pH as well as recommended rates of application of fertilizer and other amendments.

In order to remove the dense buildup of thatch created in a periodically mown field, the orchard site was burned in late December 2008. The prescribed fire also served to suppress some of the woody shrubs and vines found in the orchard site such as sericea lespedeza (*Lespedeza cuneata*) and Japanese honeysuckle (*Lonicera japonica*).

Holes for the wooden line and corner posts were drilled using a hydraulic auger. This was accomplished in approximately two hours using two people. Setting the posts took five days and used an average of 3 persons per day. One of the reasons setting the posts took such a long time is that the 18-inch auger used to drill the holes was vastly oversized for the task. For a 4-inch post, and even for the 8x6 gate posts used, it is much more efficient to use the smallest auger that will reasonably hold the post and stabilizing concrete. Because the holes were so wide, the amount of concrete needed for each post was much more than that purchased and available onsite. It was originally estimated, before the size of the holes was known, that using an 8-12 inch auger, it would take one bag of concrete to stabilize each post, roughly surrounding the post with concrete to the top of the hole. However, when placing a 4-inch post in an 18-inch hole, one bag of concrete puddles at the bottom of the hole and is only 2-3 inches thick, not substantial enough to hold the post in place. The lessons learned from this experience are: 1) make sure to add a substantial buffer onto one's concrete order, and 2) have excellent communications with all equipment operators, especially if a project manager is not onsite at the time the work is being performed.

Woven wire fencing was chosen for the orchard at VTS-Milan, as well. Erecting the fence began in mid-April, the week before the scheduled planting. Locating the orchard in a triangular field with one of the short sides already fenced with installation-standard chainlink helped to reduce the length of fence to be installed by 410 feet (820 feet of 47-inch woven wire, in total savings). A front-end loader was used to stretch the fence at Milan. Again, it was discovered that 4x4 inch posts are not strong enough to hold up to the strain of fence stretching, but in this case, unlike at the Catoosa orchard, two of the corner posts actually broke under the strain.

Replacing these posts in the midst of fencing added a considerable amount of extra time to the fence construction, but even using four persons over 5 days, the fence was still up by the day of planting.

Rows at the VTS-Milan backcross orchard were oriented so that they would run parallel to the hypotenuse of the triangularly-shaped field in order to maximize use of the well-drained portion of the site and to make mowing easier. The plan was to have the soil worked using a subsoiler attachment on the training site's tractor. This would have been especially valuable in this field setting since soils tend to get compacted over the years because of repeated bushhogging and other traffic. A subsoiler would have extended into the hard clay in the subsoil, allowing water to penetrate. However, as that particular attachment could not be located, the field was plowed instead. An attempt was made to complete two passes with the plow, in order to break the clumps of sod that had peeled to the side during the first pass; however, this idea proved ineffective and the single furrow left by the first pass was left without additional plowing.

#### Planting at VTS-Catoosa

One day prior to planting, the positions of each tree were measured off and marked with wooden stakes that would later support and anchor the 24-inch protective tree tubes. The tops of the stakes were painted different colors in order to designate which seed type went into which position within the row. This helped to minimize confusion for volunteers and to insure that orchard records are accurate.

The orchard at Catoosa, while smaller than the VTS-Milan orchard, was planted with a very diverse selection of trees. This was largely due to the fact that the supplier of both the seeds and the seedlings to be planted was able to attend the planting at Catoosa but was unable to be at the Milan planting.

It had been determined that seedlings (small trees that had been growing in a nursery setting for 1-2 years) would be planted in Rows 1 and 2, at the highest side of the orchard, and on the very last row (Row 12, on the most downhill side of the orchard). Row 1 was planted with a mixture of highly advanced backcross seedlings, D-50260181 and D-6-26-27 (both are BC3F3s, See Figure 1), along with several Allegheny chinquapin (*Castanea pumila*) controls. Allegheny chinquapins are close relatives of the American chestnut with a much smaller, shrubby growth habit. They are also susceptible to the Asian chestnut blight (*Cryphonectria parisitica*). Rows 2-12 are all planted with BC3 generation backcross seeds and seedlings. Control trees were planted at every position ending with a "1" (e.g., positions 1, 11, 21, etc.). Row 2 was planted with 2 backcross varieties: CH-102 x MAR-1 and AG-387 x TNMAC-2, both are of the BC3 generation. Rows 3-11, were planted with alternating rows of two types of BC3 seeds: AG247 x TNPOLK-1 and TNMON-8 x JB-271. Row 12 was planted with all CH-102 x MAR-1 seedlings. In all, 225 seeds and seedlings were planted at VTs-Catoosa.

Nineteen volunteers arrived to help with the planting at VTS-Catoosa on April 25, 2009. Saturdays were chosen for the plantings of both TNARNG orchards so that the maximum number of volunteers could attend. These included seven TNARNG personnel, 5 TACF volunteers, 3 students from a local university, and 4 members of the community. Volunteers loosened soil at each planting location and filled each hole with a weed-free, soiless amendment. A polyacrylamide crystal was also added to each planting hole (tradename: TerraSorb). This substance can absorb up to 200 times its weight in water, and when mixed in the soil should slowly deliver moisture to plant roots as the surrounding soil dries out. As the seeds and seedlings were planted, the 24-inch protective tube was placed either on top of the soil-covered seed, or around the seedling and pushed into the soil 2-3 inches. This tube has been shown to dramatically reduce the rate of hebivory by voles and mice who tend to uncover and eat unprotected seeds, sometimes even chewing a 2 or 3 year-old tree off at the soil line. The tree tubes are attached to the previously placed wooden stakes using zip ties. A piece of cylindrical plastic netting was placed over the

top of each tube to prevent birds from flying into the tubes and becoming trapped. Lastly, trees were watered and labeled using double-sided aluminum nursery tags. The entire planting event lasted about three hours.

#### **Planting at VTS-Milan**

The 7-foot tree spacings were marked off at the VTS-Milan orchard the day before planting. Painted stakes were used to denote which seeds were to be planted where, as at VTS-Catoosa. The three-foot stakes were pounded into the ground approximately one foot deep.

In addition to the three control types of trees that were planted in the orchard (open-pollinated, 100% American chestnut; open-pollinated, 100% Chinese chestnut; and F1 trees that are 50% American and 50% Chinese), three backcross types, each with different parentage, were planted. Controls were placed at every tenth position, starting at Position 1. This means that a control is in all positions that end in a "1" (e.g., 11, 21, 31, 41, etc.).

The VTS-Milan American chestnut backcross orchard was planted on Saturday, April 18, 2009. Twenty people participated in the planting and included ten ENV employees, two volunteers from the state chapter of TACF, and eight volunteers from the community.

Backcross pedigrees planted at the VTS-Milan orchard include TNMON-5 x M-19 and O-7 x I-11, both of which will produce 4<sup>th</sup> generation seed from the backcross breeding program (progeny will be Backcross 3, or BC3; See Figure 1), and also trees with a pedigree of AG247 x TNPOLK-1, a BC3 whose seeds will intercrossed with seeds from other BC3 to produce a tree that is 15/16 genetically American, on average, and will be highly blight resistant. In total, 570 seeds were planted at the VTS-Milan orchard.

The VTS-Milan orchard was planted with primarily seeds, with the only seedlings being the two test seedlings planted in early 2008 to test the soils for the fungal soil pathogen, *Phythopthora cinnamomi*. The same planting procedure was used at VTS-Milan as described for the VTS-Catoosa orchard planting, with one exception. The polyacrylamide crystals used at VTS-Catoosa were not added to the planting holes at VTS-Milan because soils at the Milan orchard site have a higher clay content than the soils at Catoosa, and there was concern that the extra water-holding capacity of the polyacrylamide could be a detriment at Milan, potentially causing seeds and trees to rot. The planting event at the VTS-Milan orchard took approximately six hours.

#### **Ongoing maintenance**

Regular maintenance of the two TNARNG American chestnut orchards has included mowing (especially in the field setting at VTS-Milan), weedeating fencerows, spraying herbicide within rows to reduce competition from surrounding vegetation, and watering. So far, 2009 has been relatively wet when compared to precipitation levels in 2008 and 2007. The need for supplemental watering has been minimal. Both sites are being fertilized with 30-10-10 Acid Special water soluble fertilizer at a rate of 4 teaspoons/gallon per tree.

In July, a blend of water soluble fungal spores was applied to all trees at the VTS-Milan orchard. The mixture was composed of fungal species known to form ectomycorrhizal associations with hardwood deciduous trees, some specifically with American chestnuts. Ectomycorrhiza (pl. ectomycorrhizae) are structures formed by the symbiotic relationship between certain fungi and many plants. The fungus surround the tips of plant roots and receives photosynthetically-produced sugars, while, in turn, providing moisture and nutrients to the plant. As the orchard site at Milan has not been in a forest condition for a number of years, it would be expected to have a very different mycorrhizal community than required for optimum chestnut growth. This mycorrhizal inoculant

was not added to the soils at VTS-Catoosa as that site has very recently been covered in a forest that is expected to contain a healthy mixture of mycorrhizae compatible with American chestnut.

Fertilizing and supplemental watering (when required) will continue for at least three years after new seeds are planted at each orchard. Vegetation management at the American chestnut orchards will be necessary for the duration of this project.

#### **Current Condition of Trees**

Three orchard surveys have been conducted at each site on three occasions each. Trees have been categorized in the following code:

O = no sign of germination

H = germinated and healthy

VS = seedling germinated, but very small ( $\leq$ 4" tall)

SI = sickly (some wilting or yellowing, but chance of recovery)

DY = seedling sprouted but appears to be dying (brown leaf edges)

D = dead (seed germinated and sprouted, seedling later died)

DS = dead seed (tube lifted and confirmed)

For purposes of data analysis, these categories will be grouped and simplified to designate four seed/tree conditions:

1) healthy germinants, 2) trees that sprouted and then died, 3) failed seed that did not sprout for some reason, and 4) planting locations in which a seed was planted, but at the date of survey, there is no sign of sprouting. This last category, also called "undetermined," is only relevant during the growing season that immediately follows the planting of a seed. Chestnut seeds are very short-lived in the soil, and typically rot or get eaten within one year of falling from a tree; hence, it is highly unlikely that "undetermined" seeds will sprout in the second spring after they were planted. During the last survey of the year in which any seeds are planted in the orchards, an attempt will be made to find the seeds or remnants thereof of all "undetermined" so that the cause of their failure may be determined.

Seed success has differed between the two sites (Figures 4 and 5). Catoosa had a higher percentage of germinants than Milan. The number of failed seed at Catoosa is likely much higher than this chart represents, but many seeds there have not been checked for viability. At the end of this first growing season, all "undetermined" seeds will be excavated to identify actual condition.

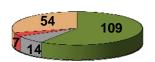








Figure 4 Seed response at VTS-Catoosa as of 1 August 2009 out of 184 seeds planted

Figure 5 Seed response at VTS-Milan as of 1 August 2009 out of 552 seeds planted

Germination rates were variable among varieties (Figures 6 and 7). Because different varieties were planted at the two sites, it is difficult to make direct comparisons; however, AG247 and TNMON x Gideon were planted at both sites and both show lower germination rates at Milan. Mortality is extremely variable between all seed types. Between backcross seedlings, mortality rates are generally low. The control varieties, however, show substantial differences in mortality rates between the 100% American & 100% Chinese. Factors influencing this

may be due to unusually high vigor in the Chinese seed stock, to the small size of the American seed stock, or to other, undetermined factors. Thus far, the F1 hybrid is performing relatively well at both orchards.

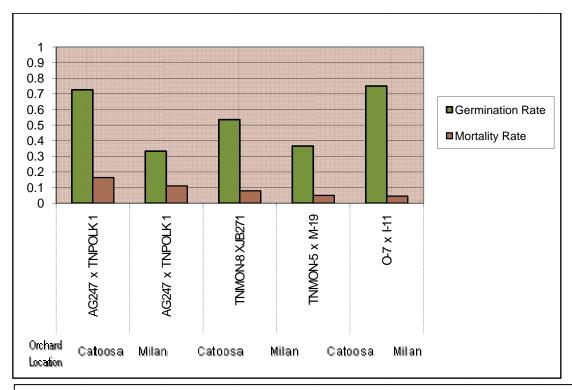


Figure 6 Germination and mortality rates of American chestnut backcross varieties planted by the TNARNG

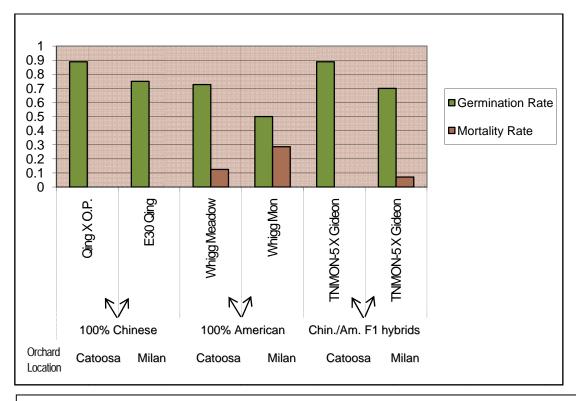


Figure 7 Germination and mortality rates of controls planted at TNARNG American chestnut orchards

There have been no signs of deer in either orchard since the gates were installed securely (this occurred very shortly following planting events). There have been some signs of vole herbivory, at both orchard locations. It appears that voles are tunneling deeper than usual to feast on a few of the planted nuts. In some cases, the seedling has survived, albeit with less energy stores than one with an unadulterated seed.

#### **Issues Encountered**

Several issues have become apparent over the course of executing this project and have been solved or overcome in a variety of ways. Here are some examples:

- Milan Drainage West Tennessee has clay-rich soils that hold water and all sites at the facility available
  for the orchard were flat. This can be a problem when growing a species that prefers well-drained soils.
  To mitigate this, we planted on the highest portion of the field, avoided all low areas, and tested the soils
  for a fungal pathogen that is sometimes found in poorly-drained soils.
- Terrasorb This polyacrylamide product was used at the VTS-Catoosa orchard to improve soil moisture availability, but may have created a barrier to seed germination and early growth. Using this material at a lower rate may eliminate this problem.
- Small diameter tree tubes The Tubex tree tubes used to protect the seeds and young seedlings are generally very effective. Tubes are purchased in nested sets of five, with the largest diameter tube (105mm) on the outside and the smallest (73mm) in the center. They prevent aboveground herbivory and help to minimize temperature extremes. However, there seemed to be a correlation between early seedling death and using the smallest diameter tubes. This observation was made while collecting tubes from trees or seeds known to be dead, when the number of small tubes removed for these reasons far outnumbered the larger tubes.
- Water availability/access The water supply for both sites must be hauled in by truck, adding to the time and effort required to water and fertilize trees. At VTS-Milan, a 250 gallon pressure sprayer is being used to deliver water to the site from the cantonment area, nearly 5 miles away. An average watering requires 3 refills of this tank and care must be taken to avoid injuring trees due to the high pressure with which water is delivered by the sprayer. A rainwater collection system is being constructed at the VTS-Catoosa orchard in which rainwater that falls on a 20 foot storage connex will be collected in a 300 gallon tank.
- Obtaining necessary materials as required by project schedule Because of procurement issues with the
  fencing materials, the planting dates for both orchards were pushed from early March, when they were
  originally scheduled, to April 18 and April 25 for Milan and Catoosa, respectively. This is slightly
  beyond the optimal planting period as it does not allow for much time for roots to become securely
  established prior to increasing temperatures and drought conditions in the summer.
- Command concerns and turnover A series of changes in command at several levels in the TNARNG resulted in setbacks in the early stages as the project had to be presented anew and approval re-gained multiple times before work could begin. A general lack of understanding of the intent of Legacy-funded projects also contributed to resistance that was encountered at some levels. The ENV office has attempted to provide a clear explanation of the goals of Legacy and of this project, in particular, and also to communicate the simple fact that the project will not be allowed to restrict training. Overall, the project has been well-received. This is likely to be an on-going concern which will be addressed through education and clarification as the need arises.

#### **Future Plans**

TNARNG orchards will be surveyed annually, noting the survival, health, and size of each tree. Once the first year's data has been collected and overall germination rates have been assessed, more trees may be added to the orchards. VTS-Milan has well over an acre of fenced land on which seeds may be planted; trees may also be added in future years at the orchards wherever a seed fails to germinate and/or wherever gaps are created by dead or culled trees.

After five to seven years of growth, the trees will be inoculated with blight to test for resistance. If trees show susceptibility to the blight, they will be culled from the orchard. BC2 trees exhibiting blight resistance will be crossed with 100% American chestnuts, while surviving BC3trees will be crossed with other BC3s from other orchards to produce the first intercross generation.



Figure 8 American chestnut backcross orchard at TNARNG VTS-Catoosa



Figure 9 American chestnut backcross orchard at TNARNG VTS-Milan